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Chemotherapeutic Trials with *Calotropis procera* Against Experimental Infection with *Theileria annulata* in Cross Bred Cattle in Pakistan

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Abstract.- The study was carried out to conduct therapeutic trials of herbal plant *Calotropis procera* and buparvaquone (Butalex) after experimental infection with *Theileria annulata* in cross bred cattle during the months of May to August, 2007 at Livestock Experimentation Station Qadirabad, Okara, Punjab, Pakistan. The experimentally infected animals developed anaemia and an enhanced inflammatory response. Mild to severe clinical reactions were recorded after experimental infection. A correlation between clinical reactions and schizont parasitosis and piroplasm parasitemia was also recorded. The animals suffered from high fever, swelling of sub mandibular and sub scapular lymph nodes, weakness, increased respiration and pulse, corneal opacity, anorexia, loss of condition, rough hair coat and incoordination. Using *T. annulata* specific primers N516/N517, 721-bp fragment of SSU rRNA was amplified from DNA of salivary glands and the internal organs of *Hyalomma* ticks. The results of therapeutic trials indicated that the characteristic macrocytic hypochromic anaemia in experimentally infected animals was recovered by *C. procera* treatment. The result of liver and kidney function tests after treatment with *C. procera* showed no toxicity at the dose rate of 0.3 mg/Kg orally (8 doses on alternate days). The efficacy of *C. procera* was higher (92.5%), compared with 75% of buparvaquone on 21 day post treatment.

Key words: *Calotropis procera*, Buparvaquone, *Theileria annulata*, Schizont parasitosis, piroplasm parasitemia, *Hyalomma*.

INTRODUCTION

Theileriosis, caused by *Theileria annulata*, is transmitted by ticks of the genus *Hyalomma.* Mortality varies from 90% in introduced exotic breeds to 5% or less in indigenous breeds. The case fatality rate of 14% has been calculated for Theileriosis at Government maintained livestock farms in Punjab, Pakistan. Mortality in fully susceptible cattle can be nearly 100 percent. In recovered cattle, chronic disease problems can occur that result in stunted growth in calves and lack of productivity in adult cattle. Out break of latently infected cattle is possible. Among several alternative *Theileria* control measures considered to date, host vaccination against *Theileria* has proved promising (Ellis *et al.*, 1996). There is currently only one effective drug for the treatment of theileriosis available in Pakistan namely buparvaquone (Butalex, ICI).

The therapy with this drug is unaffordable for farmers. Herbal plant called Ak with botanical name *Calotropis procera* R.Br. Asclepiadaceae, commonly known as milkweed or swallow-wort, is a common wasteland weed (Singh *et al.,* 1996). Ak family includes 280 genera and 2,000 species of world-wide distribution but is most abundant in the sub-tropics and tropics. This plant has been used by many researchers. Leaves of *C. procera* were used by Dolan *et al.* (1999) as antibacterial, for expulsion of guinea worms, fits in children, convulsions in children, ear ache, rheumatic joints, swellings, sores, wounds, inhibits excessive granulation of wounds and ulcers, skin diseases, killing lice, tonic, stimulant for ruminants. Basu and Nath (1999) used this plant as anti-inflammatory, anthelmintic, drastic purgative, emetic, antidote against scorpion sting, washing eyes, abortifacient, infanticide, antimplantation activity, antidote against bites of rabid dogs, antifungal. The therapeutic efficacy of *C. procera* was previously studied in a pilot study and it was found to be 80% effective against theileriosis in bovine (Durrani *et al.,* 2005). The

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present study aims at determining the efficacy of herbal plant *C. procera* on cross bred cattle and compare it with buparvaquone (Butalex) regarding control of theileriosis. It is anticipated that the result of present research will aid in the development of *C. procera* as cost effective therapy against Theileriosis in bovine.

MATERIALS AND METHODS

Experimental animals

The study was carried out during May to August, 2007 at Livestock Experimental Station, Qadirabad, Okara, and Punjab. A total of 100 cross bred cattles were selected and monitored by taking daily rectal temperatures and microscopic blood smears examination. The animals declared free from blood protozoa were retained for further study.

Rearing of infected Hyalomma *ticks for experimental infection*

Two thousand tick samples were collected from ten cross bred cattles in field cases confirmed positive for *T. annulata* by PCR. The tick samples were identified by method of Soulsby (1982). To confirm the infection of *T. annulata* in collected ticks, the semi engorged live *Hyalomma* ticks were randomly dissected for removal of salivary glands to find out number of infected acini/infected ticks by Feulgen's stain as described by Buscher and Otim (1986). Live unfed infected *Hyalomma* ticks were placed in ear bags on white rabbits and allowed to feed for 4 days before being removed for infection of experimental animals.

Infection of cattle with T. annulata

Twenty unfed infected *Hyalomma* ticks were used for infection of single cattle by applying ear bag as described by Viseras *et al.* (1999). A total of 80 animals were infected by ticks. These animals were designated as experimentally infected group. The 20 animals were not experimentally infected and hence designated as non-infected.

Collection of samples from experimentally infected animals

The blood samples were collected on day 0, 7, 14 and 21 post exposure. The severity of infection was determined on the basis of (1) rate of piroplasm infection in thin blood films: mild if less than 1%, moderate if 1-40%, severe if more than 40% piroplasms. (2) decrease in white and red blood cell count. (3) clinical reactions ranging from no reaction (no parasites and no clinical signs), mild (few schizonts, no fever or fever for 4 days otherwise clinically normal), moderate (schizont present, fever persists longer than 4 days but less than 9 days, transient clinical signs) and severe (schizont present, fever persists longer than 8 days, obvious clinical signs). Blood samples were collected in 3 ml EDTA coated vacutainers from both, infected and non-infected groups. Blood and lymph node smears were also prepared and stained with Giemsa's stain. The blood samples collected in vacutainers were processed for further use in hematological investigation.

Identification of T. annulata *in* Hyalomma *ticks*

The salivary glands and internal organs of ticks were removed as described by Chen *et al.* (2000). Genomic DNA was isolated with the help of Gentra DNA isolation kit (PURE GENE, USA, GENTRA) according to the prescribed method. The genomic DNA was used for amplification of *T. annulata* using primers shown in Table I. PCR reaction mixture contained, 1x PCR buffer, 4 mM $MgCl_2$, 0.4 mM dNTPs, 100 pmol of forward and reverse primers, 2.5 units of Taq polymerase and 0.5 µg of total genomic DNA and final volume of reaction mixture was adjusted to 50 µl with autoclaved distilled water. The PCR reaction cycling condition involves initial denaturation at 94°C for 4 minutes and then 30 cycles each comprising of denaturation at 94°C for 30 seconds, annealing at 55°C and extension at 72°C for 30 seconds followed by final extension at 72°C for 5 minutes. The samples were run on 1% agarose gel.

Restorative study

To study the efficacy of Buparvaquone (Butalex, ICI) and emulsified herb of *C. procera* as treatment of tropical theileriosis, 80 animals experimentally infected with *T. annulata* were divided into four groups each having 20 animals. Group A (no Reaction), B (mild reaction), C (moderate reaction) and D (severe reaction). Each

Table I.- Oligonucleotide primers used to detect *T. annulata* (Allsopp *et al.*, 1993).

Primers	Primer Sequence	Target position on genome	Target region	Amplicon size (bp)
N516(F)	GTAACCTTTAAAAACGT	234–250	SSU rRNA gene	721
N517(R)	GTTACGAACATGGGTTT	954–938	SSU rRNA gene	(*Theileria annulata* specific)

group is further subdivided into two groups, A1, A2, B1, B2, C1, C2, D1 and D2 with ten animals in each group. Twenty animals were kept as uninfected control. The animals of sub groups A1, B1, C1 and D1 were treated with Buparvaquone 2.5mg/Kg body weight on alternative days while animals of sub groups A2, B2, C2 and D2 were treated with homogenized buds and flower, 0.3mg/Kg orally, 8 doses on alternate days. The efficacy of drugs used in the study was determined from recovery from clinical reaction, rate of piroplasm infection in hematological observations performed on 0, 7, 14 and 21 day post medication. Liver function tests (LFT's) and kidney function tests were also performed as described in Randox blood chemistry manual, Randox Lab,UK, 21 days after drug trials with *C. procera* to determine the toxicity of herbal drug.

RESULT

Clinical signs after experimental infection

The clinical signs included high fever, swelling of sub mandibular and sub scapular lymph nodes, weakness, increased respiration and pulse, anorexia, loss of condition, in-coordination were observed. Signs of lacrimation, pale conjunctiva, and dyspnea were observed in only one animal.

Effect on haematological parameters

Giemsa's-stained blood films contained *Theileria* piroplasms including rod, signet-ring forms with diameter of 0.5-1.5 μm. Schizonts were found in blood lymphocytes in lymph node biopsy smear examination. Abnormalities in erythrocyte included anisocytosis, poikilocytosis, and basophilic strippling. The results of complete blood cell count (CBC) showed regenerative anaemia. The values of total erythrocyte count, total leukocyte count, packed cell volume and hemoglobin showed significant decrease. The correlation between Schizont parasitosis and piroplasm parasitemia is shown in Table II. Comparisons between infected and non infected animals showed a significant decrease in total leucocyte 38%, eosinophils 35%, neutrophils (31.5%) lymphocytes (33%) and monocytes count (23%) (Table III).

Table II.- Correlation of clinical manifestations with schizont parasitosis and periplasm parasitemia post infection.

Days	Clinical manifestation	Schizont parasitosis	Periplasm parasitemia
0	Mild	-	-
7	Moderate	+ (Few)	Mild (< 1%)
14	Severe	++ (More)	Moderate-severe (1- 40 %)
21	Severe	+++ (Large number)	Severe (> 40 %)

PCR amplification of T. annulata

Figure 1 shows 721-bp fragment amplified in all samples infected in *T. annulata* using N516/N517 set of primers.

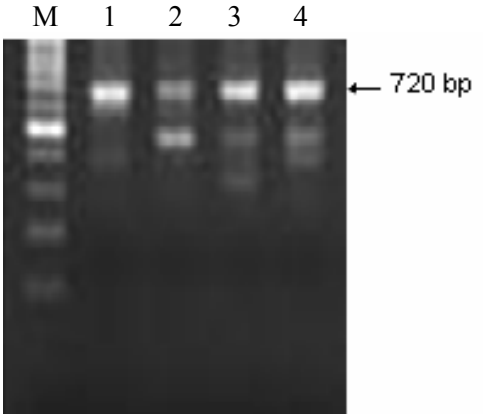

Fig. 1. PCR amplification of 721 bp fragment specific to *T. annulata* by using primer set N516/N517. M, molecular weight marker; Lanes 1-4, positive samples.

Table III.- Hematological observations on different post infection days of theileriosis.

Parameters	Non infected animals (*n* = 20)	0 day post infection (*n* = 80)	7 day post infection (*n* = 80)	14th day post infection (n= 80)
WBCs (10^3 μl^{-1})	8.41 ± 0.28	8.30 ± 0.30	5.57 ± 0.41***	5.21 ± 0.45***
Neutrophils (10^3 μl^{-1})	2.92 ± 0.31	2.89 ± 0.32	2.13 ± 0.34***	2.00 ± 0.37***
Lymphocytes (10^3 μl^{-1})	3.82 ± 0.15	3.10 ± 0.15	2.92 ± 0.15***	2.56 ± 0.17***
Monocytes (10^3 μl^{-1})	0.39 ± 0.02	0.36 ± 0.02	0.33 ± 0.03*	0.30 ± 0.04*
Eosinophils (10^3 μl^{-1})	0.17 ± 0.01	0.16 ± 0.01	0.14 ± 0.03**	0.11 ± 0.04**
RBCs (10^6/µl)	9.31 ± 0.02	7.4 ± 0.20**	6.20 ± 0.23***	5.9 ± 0.26***
Hb (g/dl)	12.51 ± 0.17	8.37 ± 0.24***	8.39 ± 0.26***	8.43 ± 0.28***
PCV (%)	37.29 ± 1.7	28.08 ± 3.2***	26.08 ± 3.5***	20.08 ± 3.8***

* P = 0.02, ** P = 0.01, *** P < 0.0001.

Table IV.- Hematological values after treatment with Buparvaquone.

Variable	Normal values	0 day post treatment (*n* = 10)	7 day post treatment (*n* = 10)	14th day post treatment (*n* = 20)	21 day post treatment
WBCs (10^3 μl^{-1})	8.41 ± 0.28	5.21 ± 0.45***	5.68 ± 0.42***	6.20 ± 0.49***	6.91 ± 0.52***
Neutrophils (10^3 μl^{-1})	2.92 ± 0.31	2.00 ± 0.37***	2.33 ± 0.34***	2.39 ± 0.35***	2.55 ± 0.39***
Lymphocytes (10^3 μl^{-1})	3.82 ± 0.15	2.56 ± 0.17***	2.92 ± 0.16***	3.0 ± 0.18***	3.4 ± 0.19***
Monocytes (10^3 μl^{-1})	0.39 ± 0.02	0.30 ± 0.04*	0.34 ± 0.06*	0.36 ± 0.07*	0.38 ± 0.08*
Eosinophils (10^3 μl^{-1})	0.17 ± 0.01	0.11 ± 0.04**	0.13 ± 0.04**	0.14 ± 0.04**	0.16 ± 0.06**
RBCs (10^6/µl)	9.31 ± 0.02	5.9 ± 0.26***	6.20 ± 0.23***	6.21 ± 0.23***	6.25 ± 0.27***
Hb (g/dl)	12.51 ± 0.17	8.43 ± 0.28***	8.92 ± 0.30***	8.93 ± 0.30***	9.02 ± 0.33***
PCV (%)	37.29 ± 1.7	20.08 ± 3.8***	23.08 ± 3.6***	24.01 ± 3.8***	24.09 ± 3.9***

* P = 0.02, ** P = 0.01, *** P < 0.0001.

Table V.- Clinical response of Buparvaquone and *Calotropis procera* treatment.

Clinical reaction type	No of animals treated	Piroplasm parasitemia (%)	No of animals cured after Buparvaquone treatment	No of animals cured after *Calotropis procera* treatment
No reaction	10	Nil	10	10
Mild	10	Less than 1	10	10
Moderate	10	1-40	7	9
Severe	10	More than 40	3	8

Restoractive efficacy of Buparvaquone

Out of 40 animals in all four sub groups 30 recovered (75 % cured clinically) indicating normal values, 6.91 x 10^3 μl^{-1}, 0.16 x 10^3 μl^{-1}, 2.55 x 10^3 μl^{-1}, 3.4 x 10^3 μl^{-1} and 0.38 x 10^3 μl^{-1} of total leucocyte count, eosinophil, neutrophil, lymphocytes and monocytes counts respectively which are very close to the normal values of these parameters as described in Table IV.

Restoractive efficacy of C. procera

Out of 40 animals in all subgroups 37 recovered (92.5% cured clinically). The clinical response of *C. procera* treatment is shown in Table V on the basis of microscopically determined periplasm parasitemia in terms of percentage. The complete blood picture of animals after treatment with *C. procera* is shown in Table VI. The Table VI indicating after treatment the values, 7.0 x 10^3 μl^{-1},

Table VI.- Hematological values after treatment with *Calotropis procera.*

Variable	Normal values	0 day post treatment(n = 10)	7 day post treatment(n = 10)	14th day post treatment(n = 20)	21 day post treatment
WBCs (10^3 μl^{-1})	8.41 ± 0.28	5.21 ± 0.45***	5.69 ± 0.42***	6.25 ± 0.50***	7.0 ± 0.56***
Neutrophils (10^3 μl^{-1})	2.92 ± 0.31	2.00 ± 0.37***	2.36 ± 0.36***	2.40 ± 0.37***	2.58 ± 0.40***
Lymphocytes (10^3 μl^{-1})	3.82 ± 0.15	2.56 ± 0.17***	2.92 ± 0.16***	3.14 ± 0.18***	3.46 ± 0.20***
Monocytes (10^3 μl^{-1})	0.39 ± 0.02	0.30 ± 0.04*	0.36 ± 0.07*	0.37 ± 0.07*	0.38 ± 0.08*
Eosinophils (10^3 μl^{-1})	0.17 ± 0.01	0.11 ± 0.04**	0.13 ± 0.04**	0.14 ± 0.04**	0.16 ± 0.06**
RBCs (10^6/µl)	9.31 ± 0.02	5.9 ± 0.26***	6.25 ± 0.26***	6.66 ± 0.27***	7.01 ± 0.32***
Hb (g/dl)	12.51 ± 0.17	8.43 ± 0.28***	8.99 ± 0.31***	9.03 ± 0.38***	9.10 ± 0.37***
PCV (%)	37.29 ± 1.7	20.08 ± 3.8***	24.01 ± 3.7***	24.05 ± 3.9***	24.09 ± 4.0***

* P = 0.02, ** P = 0.01, *** P < 0.0001.

Table VII.- Comparative efficacy of buparvaquone and *Calotropis procera* in treatment of theileriosis in cattle.

Drug administered	Efficacy (%)				T value
	Day 0	Day 7	Day 14	21	
Buparvaquone	10	20 (n=20)	27 (n=30)	30 (n=40)	**9.3***
Calotropis procera	10	20 (n=20)	29 (n=30)	37 (n=40)	

* Significant difference.

Table VIII.- Effect of *Calotropis procera* on different biochemical parameters of animals after 21 days treatment.

Parameter	Normal range	After treatment
ALT (SGPT) (U/L)	6.9-35	34
AST (SGOT) (U/L)	45-110	114
Alkaline phosphatase (U/L)	18-153	49
Uric Acid (mg/kg /day)	1-4	1.2
Creatinine (mg/dl)	0.6-1.8	0.7

0.16 x 10^3 μl^{-1}, 2.58 x 10^3 μl^{-1}, 3.46 x 10^3 μl^{-1} and 0.38 x 10^3 μl^{-1} of total leucocyte count, eosinophil, neutrophil, lymphocytes and monocytes counts respectively which are very close to normal values of these parameters. Ticks were not present on the body of animals after treatment. After completion of treatment with *C. procera* no tick infestation was seen. The animals showed diarrhoea for first 10-12 hours after administration of every dose of *C. procera* on alternate days but animals recovered spontaneously without any antidiarrhoeal treatment. Comparative efficacy of two drugs is shown in Table VII and results indicated that percentage efficacy of *C. procera* was comparatively high as compared to buparvaquone after 14 and 21 days of treatment. The results of liver and kidney function tests showed normal values after 21 days of treatment with *C. procera* @ 0.3 mg/Kg in oral doses as indicated in Table VIII. The values of ALT, AST, AP, uric acid and creatinine were found to be 34 U/L, 114 (U/L), 49 (U/L), 1.2 (mg/kg/day) and 0.7 (mg/dl) which are very close to normal values Table VIII.

DISCUSSION

Experimental infection with *T. annulata* was done in experimental cross bred cattle at Livestock Experimental Station Qadirabad, District Okara during the months of May, June, July and August 2007 by infected hyalomma ticks using ear bags. Similar methodology for experimental infection was used by Viseras *et al.* (1999) who were able to experimentally transmit *T. annulata* to a naïve calf with *H. lusitanicum* ticks fed on infected cattle. Salih *et al.* (2005) Talukdar *et al.* (1999), Kumar and Malik (1999), Stockhom *et al.* (2000) also experimentally induced theileriosis. Giemsa's-stained blood films contained *Theileria* piroplasms, including rod, signet-ring forms with diameter of

0.5-1.5 µm. The ring form was found to be the most common in present study. The staining character, morphology (more than 70 % ring form) and size of the parasite within the erythrocytes was considered as the best criteria to identify the organism at species level in this study and is in line with the previous researchers *i.e.* Hennings (1949) and Levine (1985). The present study revealed that the species responsible for theileriosis in cattle is *T. annulata* and the biological vector for the transmission identified by PCR test was *Hyalomma* tick which endorses the result of Ashfaq and Razzak (1983), Venkatraman *et al*. (1999) and Kawazu *et al.* (1999). The specificity of the *T. annulata* primer set N516/N517 was examined with DNA extracted from blood samples collected from experimental animals. The expected 721-bp fragment was generated from *T. annulata* DNA .The specificity was confirmed by positive controls of *T. annulata*. These findings are in accordance with Collins *et al.* (1999). The expected 721-bp fragment was detected by electrophoresis. These findings are in accordance with Oliveira and Jongejan (2005) and Roy *et al*. (2001).

The results of hematological observation showed regenerative anaemia, presence of many *Theileria* piroplasms, and neutrophilia with mild left shift, lymphocytosis, and monocytosis. The findings are in accordance with findings of Conrad *et al.* (1985), Dhar *et al.* (1986) and Mishera *et al.* (1983). Goddeeris *et al.* (2005) also recorded significant difference in total leukocyte count, red blood cell count, hemoglobin concentration and packed cell volume in cattle, similar changes have been reported previously by Omer *et al.* (2002) and Sakyi *et al.* (2004). The observed anaemia may result from intra-erythrocytic piroplasms Preston *et al.* (1992). Hooshmand-Rad (1976) attributed the resulting anaemia to an auto-immune reaction, whereas, Boulter and Hall (2000) attributed it to the erythro-phagocytosis rather than parasite-induced lysis. A significant decrease in monocyte percentage was during the present study which is in accordance with the results of Omer *et al.* (2002) in cattle but differing from those reported previously by Forsyth *et al.* (1999) who recorded a significant increase in monocytes count in their infected cattle.

All the infected animals showed clinical manifestations of high fever as the first manifestation followed by swelling of submandibular and sub scapular lymph nodes, weakness, increased respiration and pulse, anorexia, loss of condition. Corneal opacity was observed in severe cases of the disease. Neurologic sign of incoordination were also seen in five weak animals which in accordance with the findings of Srivastava and Khan (1994), Manickan and Dhar (1984), Gill *et al.* (1977) and Ashfaq and Razzak (2000). The current findings are not in accordance with Friedhoff (1999) who reported swelling of lymph nodes as the first sign in all calves along with lacrimal and nasal secretions. Osman and Al-Gaabarya (2007) also reported eye lesions which were observed in the present study. Haemoglobinaemia and haemoglobinuria were not so marked manifestations seen in the present study. The correlation between clinical reactions and schizont parasitosis as well as piroplasm parasitemia is observed during the present study. The estimation of schizont parasitosis is subjective and may reflect variations in distribution of schizont within and between lymph nodes. Hence the direct correlation between schizont parasitosis and severity of infection is not always found.

Efficacy of buparvaquone was earlier reported by Musisi et al (1996) and Mbogo et al. (1997). Osman and Al-Gaabarya (2007) studied the effect of buparvaquone treatment on the levels of some antioxidant vitamins, lipid peroxidation and glutathione peroxidase in cattle with Theileriosis. They concluded that the levels of LPO in plasma and erythrocyte samples were significantly ($P < 0.05$, $P < 0.01$) higher after treatment than in either control animals or before treatment. Kinabo and Bogan (1988) concluded that buparvaquone can be reserved for the treatment of clinical cases, in cattle of all ages. The results of present study also suggest that chemotherapy with buparvaquone is more effective in mild (early stage) of the disease as compare to moderate and severe forms (later stage). Similar findings were made by Mbwambo *et al.* (2006) and Mchardy *et al*.(1985). Emulsified buds and flowers of *C. procera* was given at dose rate of 0.3 mg/ Kg dose orally. Eight doses on alternate days. The results indicated that the characteristic macrocytic hypochromic anaemia in Theileriosis

was recovered by *C. procera* treatment and higher efficacy of *C. procera* was recorded in all forms of the disease.

This work represents a preliminary screening study and dose response studies are required both *in vivo* and *in vitro* as well as toxicity and feasibility studies before these combinations can be considered to be realistic options for application to the cattle. The identification of *C. procera* used in present study was also in accordance with Rastogi and Singh (1991) and Rajhy *et al.* (2003). Keeping in view the above work on the therapeutic effect of *C. procera* against ticks it is an important finding and cost effective treatment as there is potential loss of enzootic stability to tick-borne diseases due to potential loss of efficacy of the existing acaricides on account of increasing frequency of ticks resistant to acaricides. In addition for many resource-poor livestock owners, the newer acaricides are relatively much more expensive than those that were used in the past. The other therapeutic effects of *C. procera* were reported by Zafar *et al.* (2005) who evaluated the anthelmintic activity of *C. procera* .The result of liver and kidney function tests post treatment with *C. procera* showed normal values indicating no toxicity at the dose rate of 0.3 mg/ Kg dose orally. The statistical analysis showed significant difference in the efficacy of two drugs. The results of present study showed that after completion of treatment with *C. procera* no tick infestation was seen. After treatment with *C. procera* the levels for alkaline phosphate and AST value was in normal range showing absence of toxicity due to drug and recovery of animal which is further endorsed by Rick (1999) who noted that during the course of infection of calves with *T. annulata* levels of alkaline phosphatase increase while Stockham *et al.* (2000) also reported increased serum aspartate transaminase (AST), γ-glutamyl transferase (GGT), and creatine kinase (CK) activities.

It is concluded from above discussion that knowledge of cattle population susceptibility is clearly vital before choosing the disease control programme. In addition, for the treatment to be effective it must be applied early in the course of the disease. Such prompt actions will require a good diagnostic ability, both sensitive and specific, and high standard of management by the farmers. Anti-Theilerial drugs should be available on a long term basis and the treatment should be affordable. Butalex being a costly medicine is not affordable to majority of farmers with stocking pattern of 1-2 large animals hence the novel treatment of Theileriosis and control of ticks with *C. procera* is an alternative which is affordable. In Pakistan, *C. procera* flowers are mostly used as an anthelmintic in small ruminants in the form of decoction and/or crude powder mixed with jaggery and administered as physic drench/balls. Further research in order to study the efficacy of this herbal medicine in cell based system and HPLC profiling of the product with the objective to develop this herbal medicine for manufacturing by private sector is required.

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